A Rat Model of Early Sepsis: Relationships between Gentamicin Pharmacokinetics and Systemic and Renal Effects of Bacterial Lipopolysaccharide Combined with Interleukin-2

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A rat model of early sepsis induced by lipopolysaccharide (LPS) combined with interleukin-2 (IL-2) was developed. The primary aim was to assess the pharmacokinetics of gentamicin and sepsis-induced pathophysiological changes. Moreover, the effects on the glomerular filtration rate and tubular function were studied in septic and control rats. First, an intravenous (i.v.) bolus of LPSIL-2 (1 mg/kg-Pseudomonas aeruginosa, 15 µg/kg IL-2) or saline (controls, C) was administered. The Wistar rats were treated 30 min after LPSIL-2 with gentamicin as a 3 mg/kg i.v. bolus followed 10 min later by an i.v. 170-min infusion (GE, 0.09 mg/kg·min⁻¹). The monitoring of vital functions, biochemistry and GE concentrations was performed. Creatinine clearance was 2–3 times lower and fractional urine excretion was 3–4 times less in septic rats as compared to controls (p < 0.05), although urine flow was comparable. Capillary leakage caused a 55% elevation in the volume of distribution (V) in the LPSIL+GE group vs. C+GE (p < 0.05). The renal CLige was less (2.2 ± 0.59 vs. 3.8 ± 0.53 mL/min·kg⁻¹, p < 0.05), while the total CLige was comparable (5.9 ± 1.5 vs. 6.7 ± 1.1 mL/min·kg⁻¹; p = 0.30). In the LPSIL+GE group relative to C+GE, the half-life (t1/2) was 79% higher (p < 0.05) and GE concentrations detected at the end of the study in the plasma and kidney were elevated 2.5-fold (p = 0.09) and 2.2-fold (p < 0.05), respectively. The model reproduced several consequences of early sepsis like in patients such as capillary leak, a decreased glomerular filtration rate (GFR) and the changes in pharmacokinetics of GE (increased values of V and t1/2 and a drop in renal CLige proportional to that of CLige). Nonrenal routes which, for the most part, compensate the reduced renal CLige in septic rats deserve further study.

Key words: gentamicin; sepsis; pharmacokinetics; LPSIL-2; early endotoxemia; multi-organ injury

Sepsis remains a major cause of death in the intensive care unit.1,2) The development of acute kidney injury (AKI) during sepsis adversely affects the function of other organs and results in higher morbidity and mortality. AKI is detected and classified using an increase in serum creatinine with or without a concomitant drop in urine output, i.e. the biomarkers of a sudden decrease in the glomerular filtration rate (GFR).3,4) Administering drugs and life-saving antibiotics is particularly difficult in critically ill patients with sepsis. The distribution and elimination of the drug are subjected to continuous changes which depend on cardiac output, glomerular and tubular functions, hepatic metabolism, fluid retention, albuminemia, and other factors. Moreover, the inter-individual variability in pharmacokinetics is significantly increased due to the variable severity of the disease.5,6)

Aminoglycosides are antibiotics with concentration-dependent bactericidal effects and are commonly used to treat sepsis caused by Gram-negative (G-) bacteria. Despite a considerable risk of nephrotoxicity and ototoxicity, gentamicin (GE) is still frequently used due to its fast effect, low resistance and low cost. The drug rapidly distributes in the plasma and interstitial fluid and its enlarged distribution caused by capillary leak and tissue edema induced by sepsis may reduce its maximum concentrations and negatively affect the outcome of the therapy. The major elimination route for GE is renal excretion of unchanged drug. Renal clearance of GE depends on glomerular filtration rate (GFR) which is significantly reduced in septic patients. This may result in accumulation of the drug and toxicity. GE and other aminoglycosides have a narrow therapeutic range. Therapeutic monitoring of GE concentrations and individualized dosing are strongly recommended in septic patients.

The signs of GE nephrotoxicity include nonoliguric or polyuric renal dysfunction accompanied by an increase in plasma creatinine and other metabolic products, and an increased urinary loss of glucose, amino acids, proteins, enzymes, calcium and magnesium.6,7) Traditionally, the nephrotoxicity of GE has been considered a tubulopathy in which tubular damage is the principal mechanism of AKI, resulting in reduced renal blood flow and GFR via tubulo-glomerular feedback mechanisms.5,8) However, under the condition of therapeutic drug monitoring, massive overdosing with GE leading to cases of severe tubular injury is less frequent, although plasma creatinine and urea can still increase as markers of a reduced GFR. Evidence has accumulated showing that GFR reduction caused by GE can be ascribed to mesangial and vascular contraction caused by both direct and indirect mechanisms.8–10)

In this study, a rat model of early sepsis induced by low-dose bacterial lipopolysaccharide (LPS) combined with interleukin-2 (IL-2) was developed and characterized. The primary aim was to assess the pharmacokinetic properties of GE in relation to pathophysiological changes induced by

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sepsis. Moreover, the effects of therapeutic concentrations of GE were studied in septic and control animals on the GFR and tubular function. GE was selected as a typical example of drugs with clinically important changes in pharmacokinetics and pharmacodynamics occurring in sepsis. Animal models are valuable for better understanding of sepsis-induced pathophysiological changes relevant to pharmacokinetics and pharmacodynamics. Such knowledge could help to optimize GE dosing to septic patients if successfully transferred from animal to human conditions.

MATERIALS AND METHODS

Animals Specific pathogen-free (SPF) male Wistar rats (320–350 g body weight) were supplied from Biotest s.r.o., Konárovice (Konárovice, Czech Republic). The animals received humane care according to the ethical standards of Directive 86/609/EEC, “European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes” (1986), and the “Guiding Principles in the Use of Animals in Toxicology” (1989). The study was approved by the Animal Welfare Committee of the Charles University in Prague, Faculty of Medicine in Hradec Kralove. The rats were housed in standard boxes and provided with standard chow and sterilized water ad libitum and maintained at a 12-h light/dark cycle.

Chemicals Bacterial LPS from *Pseudomonas aeruginosa* (Cat. No. L 9143), recombinant mouse IL-2 (Cat. No. I 0523), pentobarbital sodium salt (Cat. No. P 3761) and gentamicin (Cat. No. G 3632) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sterile saline for infusions was supplied by B. Braun Medical s.r.o. (Prague, Czech Republic).

Animal Preparation The rats were anesthetized with an intraperitoneal (i.p.) injection of pentobarbital sodium (50 mg/kg) and subjected to laparotomy through a small midline incision under aseptic conditions. A urinary bladder catheter was positioned and then the abdominal cavity was closed. The rats breathed spontaneously and were kept at a rectal temperature of 37.5°C with the help of a feedback-controlled heating system (Heating Pad, P-Lab a.s., Prague, Czech Republic). Blood was drawn from a catheter in the left femoral artery. Another catheter was inserted into the right jugular vein for intravenous (i.v.) bolus dosing and continuous infusion was performed with a syringe pump Perfusor Compact (B. Braun Medical s.r.o., Prague, Czech Republic). An electrocardiogram (ECG) was observed continuously and recorded every 20 min. Heart rate (HR) and arrhythmias were assessed (Animal BioAmp ML136, LabChart software, ADInstruments GmbH). At 20-min periods, the respiratory rate (RR) was monitored invasively through a tracheal catheter with the help of Spirometer ML141, ADInstruments GmbH, Spechbach, Germany.

Model Validation The rats underwent all the procedures described above and were given i.v. bolus doses of LPS (range: 0.1–2 mg/kg) and IL-2 (range: 5–30 µg/kg) in order to mimic early sepsis with capillary leakage. The extent of systemic microvascular leakage was evaluated using the hematocrit, albuminemia and white blood cell count (WBC).11,12) The animals were sacrificed by exsanguination and the lungs, liver, kidneys, heart and spleen were removed and their wet weight was determined. The organs were fixed and rinsed for 14 d in Baker’s solution (100 mL formaldehyde 38%, 900 mL tap water, and 10 g calcium chloride). After 24 h of fixation, the lungs were dried, weighed, and refixed. The samples were rinsed at least two hours in running tap water, dehydrated with ethyl alcohol and acetone in a histological processor for 24 h, cleansed with xylene, and soaked in paraffin. Sliding microtomes were used with steel knives for cutting 6 µm thick sections from the paraffin blocks. Sections on slides were deparaffinized in xylene and alcohol, rehydrated, and stained with hematoxylin and eosin. Sections of kidneys and spleen were stained with orcein and resorcin-fuchsin as well. Digital photographing was made with light microscope Olympus BH-2. Microphotographs were archived with the help of Olympus MicroPhoto v2.2 software.

Study Design and Treatments The rats (n=28) were randomly assigned to four experimental groups of seven and were subjected to the following treatments:

- **Group 1 (C+Sal):** Controls injected with saline and treated with saline.
- **Group 2 (C+GE):** Controls injected with saline and treated with gentamicin (GE).
- **Group 3 (LPSIL+Sal):** Rats injected with LPS and IL-2 and treated with saline.
- **Group 4 (LPSIL+GE):** Rats injected with LPS and IL-2 and treated with GE.

The time schedule of the study was as follows (Chart 1):

1. Stabilization, challenge and endotoxemia development (0–60 min): a 30-min equilibration period after surgery with blood sampling for baseline values of laboratory tests at min 30 was followed by LPSIL-2 (Groups 3 and 4) or saline (Groups 1 and 2) injection at 30 min and by another 30-min period of endotoxemia development;
2) Treatment (60–240 min): an i.v. bolus injection of GE (3 mg/kg i.v., Groups 2 and 4) or saline (Groups 1 and 3) was administered at 60 min. Ten minutes later, a 170-min i.v. infusion of GE in saline (0.05 mg/kg-min⁻¹, Groups 2 and 4) or saline only (Groups 1 and 3) was begun. The infusion was terminated at 240 min. Blood for laboratory investigations was taken at 210 min and urine was collected between 60 min and 240 min;

3) Follow-up (240–420 min): a 180-min period of continuous body fluid sampling and monitoring of vital functions. Blood for laboratory investigations was drawn at 390 min and urine was collected over the entire follow-up period.

**Laboratory Examination** Routine clinical biochemistry and hematology tests were performed at the Department of Clinical Biochemistry and Diagnostics and the Department of Hematology at the Teaching Hospital in Hradec Kralove. Acid-base parameters such as pH, pO₂, pCO₂, HCO₃⁻ and the actual base excess (aBE) were analyzed in arterial blood samples (140 µL each) using a Stat Profile Critical Care Xpress, NOVA Biomedical Corp., (Tecom Analytical Systems CS s.r.o., Prague, Czech Republic). Blood samples were taken at 210 min and urine was collected over the entire follow-up period.

Creatinine clearance (CLₐr) was calculated as the amount of creatinine excreted per unit of time during the collection interval of 180 min divided by its plasma concentration:

$$CL_{r} = \frac{\left(U - crea \times V_{urine}\right)}{(P - crea \times 0.001)} / 180 \text{ min}$$

where $V_{urine}$ is the volume of urine [mL], $U - crea$ [mmol/L] and $P - crea$ [µmol/L] are urinary and plasma concentrations of creatinine. Fractional excretion of urea ($FE_{urea}$) was calculated using the formula:

$$FE_{urea} \% = \frac{100 \times (U - urea / P - urea)}{(U - crea / (P - crea \times 0.001))}$$

where $U - urea$ [mmol/L] and $P - urea$ [mmol/L] are urinary and plasma concentrations of urea nitrogen, respectively. Fractional excretion of water ($FE_{H₂O}$) was obtained as follows:

$$FE_{H₂O} \% = \frac{100 \times (P - crea \times 0.001)}{U - crea}$$

**Gentamicin Assay** Gentamicin concentration was measured in the plasma, urine and kidney homogenate using a fluorescence polarization immunoassay (AxSym gentamicin reagent pack) and an AxSym autoanalyzer (Abbott Laboratories s.r.o., Prague, Czech Republic). Blood samples were collected at 3, 6, 9 min after the i.v. bolus, then at 30, 120, and 170 min of i.v. infusion, and at 180 min following its completion. Samples were kept at 2–6°C. Plasma was separated by centrifugation (3000 × g, 4°C, 10 min), frozen and stored at −80°C until analysis. Kidneys removed from the animal were weighed and immediately deep frozen in liquid nitrogen. Homogenization was performed manually in ceramic mortar using liquid nitrogen. Powdered frozen tissue was weighed (0.2 g), suspended in citrate buffer (1 mL, pH 4), vortexed and centrifuged at 10000 × g at 4°C for 10 min. The supernatant was then stored in 150 µL aliquots at −80°C until analysis.

**Pharmacokinetic Analysis** After a bolus dose of GE, the volume of distribution of the central compartment ($V_c$) was estimated using the formula $V_c = D / C_{eq}$, where $C_{eq}$ is the concentration extrapolated to time zero using the linear regression analysis of the relationship between the natural logarithms of the GE concentrations observed at 3, 6 and 9 min and the administered dose ($D$). Total plasma clearance ($CL$) of GE was calculated using the steady-state formula: $CL = R_{inf} / C_{eq}$, where $R_{inf}$ is the infusion rate and $C_{eq}$ is the steady-state concentration of plasma GE. The half-life was calculated as follows: $T_{1/2} = LN(2) / V_{c} / CL$. Renal clearance of GE was estimated using the formula $CL_{R} = A_{r} / AUC$, where $A_{r}$ is the amount of GE excreted in urine and $AUC$ is the area under the curve–plasma concentrations of GE calculated over the urine collection interval with the help of the linear trapezoidal rule.

**Statistical Analysis** The statistical analyses were performed using GraphPad Prism v5.02 (GraphPad Software Inc., SD, California, U.S.A.). Between-group differences were analyzed using a two-way analysis of variance (ANOVA) followed by Bonferroni post hoc tests. Within-group comparisons were done using a paired t-test or one-way analysis of variance for repeated measures followed by the Tukey–Kramer multiple comparison tests. A value of $p<0.05$ was considered significant. All values are expressed as means±S.D. unless indicated otherwise.

**RESULTS**

**Model Development** Based on preliminary experiments, a combination of 1 mg/kg of LPS and 15 µg/kg of IL-2 was selected. The mortality rate 72 h after injection of these doses of LPSIL-2 was zero from a total number of 25 animals studied as an additional group during the model development phase. In the endotoxemic animals, the following changes occurred at 30 min after administration of LPSIL-2 as compared to the baseline: HCT raised from 0.43±0.03 to 0.46±0.03 (p<0.001), albuminemia decreased from 35.0±3.0 to 31.2±4.1 g/L (p<0.05) and WBC decreased from 5.4±1.1×10⁹/L to 2.5±0.8×10⁹/L (p<0.01). No significant changes in either HCT (0.43±0.03 vs. 0.42±0.03, p=0.055), albuminemia (36.4±1.4 vs. 34.8±2.0, p=0.055) or WBC (4.0±0.6 vs. 5.4±1.7×10⁹/L, p=0.2) were observed in the control animals. The baseline heart rate was similar in both groups (436±36 vs. 431±42 min⁻¹, p>0.05). The heart rate was stable over the first 180 min interval following the administration of LPSIL-2. Thereafter, it gradually increased to a maximum value of 481 min⁻¹ observed at 360 min (a 10% increase from the baseline, p<0.01). In contrast, serial measurements in the controls documented either no change or only slightly decreased heart rate by 10% (p<0.05) at several intervals. Arrhythmia episodes were not observed. A gradual increase in the respiratory rate was observed in the endotoxemic group from 109±5 (the baseline) to 123±5 (60–240 min) and to 135±8 breaths/min (60–240 min) (p<0.001). No such changes were found in the control group (118±5 vs. 109±5 vs. 112±11 breaths/min, p=0.07). Sections of kidneys, heart, inner ear, and brain from rats injected with LPSIL-2 showed no morphological differences when compared to controls. Notably, remarkable pathological changes were found in lung (Fig. 1) and spleen specimens. The erythrocyte counts were significantly increased in the red pulp of the spleen. Erythrocytes lavishly infiltrated white pulp as well (data not shown). The wet weight of the lungs (2.91±0.28 vs. 2.28±0.21 g; p<0.005) and spleen (0.90±0.09 vs. 0.69±0.13 g; p<0.05) were significantly higher in the endotoxemic group when compared to controls, whereas other organs (liver, kidneys and heart) had comparable weights (data not shown).

**Biochemical Parameters** The baseline values of all
but one biochemical parameter were comparable in all four groups. The only exception was $pO_2$, which differed slightly between groups but showed adequate oxygenation in all of them (Table 1). Changes in acid-base parameters ($pH$, $pCO_2$, and $abe$) corresponding to the development of lactate acidosis were compensated by hyperventilation leading to hypocapnia. Throughout both study periods, $pCO_2$ and $abe$ gradually decreased in all groups. However, a much greater change was seen after administering LPSIL-2 than in the saline-injected controls (C+Sal and C+GE). In all groups, adequate blood oxygenation was maintained. The $pH$ was either stable or slightly increased (Table 1). Plasma lactate was not changed in the C+Sal group, with only a transient elevation detected in the C+GE or LPSIL+GE groups. In contrast, the LPSIL+Sal group exhibited a sustained increase of plasma lactate, which was 2.6-fold elevated in the follow-up period when compared to the baseline. The increase in the activity of AST (3.6-fold above the baseline) was higher in the LPSIL+Sal and LPSIL+GE groups as compared to the C+Sal (1.7-fold) and C+GE (1.4-fold) groups (Table 1). Plasma creatinine and blood urea nitrogen (BUN) continuously increased in both groups of LPSIL-2-challenged animals to levels 2–3 times higher (creatinine) and 2.5 times higher (BUN) during the follow-up as compared to the baseline. In control rats treated with saline or GE, the extent of the increase was only 50% and 30% for creatinine and BUN, respectively (Table 1).

Renal Function
No between-group differences were found in the flow rate of urine collected over the treatment and follow-up periods. In the LPSIL+GE group, the flow rate decreased during the follow-up as compared to the treatment period. In the C+Sal group, its value was stable in time and a trend towards a reduction was observed in the C+GE and LPSIL+Sal groups (Table 2). In the groups challenged with LPSIL-2 (LPSIL+Sal and LPSIL+GE), the $CL_{cr}$ observed during the treatment period was less than in the other two groups and further decreased during the follow-up. This decrease was not seen in the C+Sal and C+GE groups. Thus, the groups injected with LPSIL-2 had approximately 2 to 3 times lower $CL_{cr}$ during the follow-up period than the C+Sal and C+GE rats. Notably, administering GE did not influence the $CL_{cr}$ in either the controls or the LPSIL-2 rats (Table 2). In the treatment period, no differences were found between groups in either the excreted urea or the fractional excretion of urea ($FE_{UN}$). In the groups injected with LPSIL-2, however, both characteristics of urea excretion were significantly reduced during the follow-up period by 40–65% as compared to the treatment period. In rats not exposed to LPSIL-2, urea excretion remained stable (C+GE) or increased (C+Sal), while $FE_{UN}$ was markedly augmented (Table 2). As a result, the LPSIL-2 rats showed a 3 to 4-fold reduction in urea excretion and $FE_{UN}$ during the follow-up as compared to the other groups. During the treatment period, GE treatment had no effect in rats injected with LPSIL-2, whereas decreased urea excretion was found in controls. The $FE_{UN}$ was not influenced by GE in either the controls or the LPSIL-2 rats (Table 2). The fractional excretion of water did not differ between groups in the treatment period. Its highest value was detected in the LPSIL+Sal group during the follow-up but the difference was only modest (Table 2).

Pharmacokinetics of Gentamicin
Arithmetic mean (SEM) plasma concentrations of GE after an i.v. bolus dose followed by an i.v. infusion of the drug are shown in Fig. 2. Pharmacokinetic characteristics are summarized in Table 3. The concentration at the first sampling interval of 3 min following the i.v. bolus dose was 19% higher in controls than in LPSIL-2 rats, although the difference did not reach significance ($p=0.2$). An initial decrease in the GE plasma concentration over the first 10 min followed first-order kinetics. The log-transformed concentrations fitted well to a least-square line with the coefficient of determination $r^2$ exceeding 0.8 in all animals. The derived volume of distribution of the central compartment ($V_c$) was 55% larger in the LPSIL+GE rats than in the C+GE group ($p<0.05$). Repeated-measures ANOVA showed that a steady-state plasma concentration of GE was maintained in both groups from min 130 of the infusion until its end. LPSIL-2 caused no significant changes in either the total plasma $CL$ of GE or in the steady-state plasma concentration of GE ($C_{ss}$). The total plasma $CL$ of GE was only marginally less and $C_{ss}$ was higher in the LPSIL+GE rats than in the C+GE group (Table 3). In contrast, renal $CL$ of GE significantly decreased (42%, $p<0.05$) in proportion to $CL_{cr}$ (30% decrease, Table 2). Consequently, the ratio of gentamicin renal clearance ($CL_{rg}$) to $CL_{cr}$ was similar in both groups (Table 3). Furthermore, a simultaneous analysis of both groups showed that renal $CL$ of GE was well correlated with $CL_{cr}$ ($r=0.67$, $p<0.01$). The renal $CL$ of GE accounted for 57% of the total plasma $CL$ of GE in the C+GE group but for only 36% in the LPSIL+GE ($p=0.079$, Table 3), thus providing evidence that nonrenal clearance mechanisms were more important in the latter group. The plasma half-life of GE was 79% higher ($p<0.05$) in the LPSIL-2 group than in controls. Moreover, GE concentrations detected at 360 min in the plasma and kidney tissue homogenate were 2.5-fold ($p=0.09$) and 2.2-fold.
The drug mainly distributes in the volume of plasma and interstitial fluid. The increase in $V_c$ is therapeutically relevant because this pharmacokinetic characteristic influences the maximum concentration of GE after a short-term (30 min) i.v. infusion used in clinical practice. Furthermore, the drug exhibits a concentration-dependent bactericidal effect.

Most published studies with rats have documented declines in renal blood flow and GFR after GE dosing similar to that used in the classical models of GE-induced severe tubular injury (50–100 mg/kg daily for several days), *i.e.* after massive overdosing. In the range of therapeutic steady-state concentrations, GE did not reduce $CL_{cr}$ (the marker of GFR) either in controls or LPSIL-2 rats. This is in contrast to its marked decrease due to the effect of LPSIL-2 alone. Presumably, the GFR-reducing effect of GE described in the literature requires an exposure to higher concentrations of the drug over a longer period of time than that used in this study (6h). In concordance with this view, Ngeleka *et al.* did not observe any GE-mediated alteration of functional and histologic parameters in rats after 5 or 8d of dosing with a daily dose of 15 mg/kg, *i.e.* a lower dose than that used in the models of tubular toxicity. However, measurable abnormalities of the kidneys were found in endotoxemic animals injected with

### DISCUSSION

The present study describes, for the first time, a rat model of early sepsis induced by a bolus injection of LPS combined with IL-2. Both the separate and combined effects of LPSIL-2 and GE on GFR, tubular function and plasma biochemical parameters were evaluated. Importantly, therapeutic steady-state concentrations of GE were maintained throughout the experiments. At the same time, pharmacokinetic monitoring made it possible to evaluate the influence of both capillary leakage and altered renal function on GE disposition. Albright different from therapeutic protocols used in clinical practice, administration of GE as an i.v. bolus followed by 170-min i.v. infusion was advantageous because the $V_c$ of the drug could be estimated accurately, while both the clearances of GE and $CL_{cr}$ could be measured during the infusion.

Administration of a low-dose LPS from *Pseudomonas aeruginosa* combined with IL-2 to adult Wistar rats induced the following: capillary leakage documented at min 30 to 45 after injection by increased hematocrit, decreased albuminemia, leucocytopenia and a 55% elevation in the $V_c$ of GE.

The table below shows the results of clinical biochemistry tests:

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline (B)</th>
<th>Treatment (T)</th>
<th>Follow-up (F)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO₂ [kPa]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>5.3±0.3</td>
<td>4.6±0.6</td>
<td>4.4±0.5</td>
<td>F, T vs. B</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>5.2±0.7</td>
<td>4.7±0.7</td>
<td>4.4±0.7</td>
<td>F vs. B</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>5.7±0.6</td>
<td>4.7±0.4</td>
<td>3.7±0.4</td>
<td>F, T vs. B, F vs. T</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>5.5±1.1</td>
<td>3.7±0.3</td>
<td>3.4±0.2</td>
<td>F, T vs. B</td>
</tr>
<tr>
<td>pO₂ [kPa]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>9.6±0.7</td>
<td>9.8±0.9</td>
<td>10.2±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>11.2±1.0</td>
<td>12.1±1.3</td>
<td>11.4±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>9.8±1.3</td>
<td>10.1±0.8</td>
<td>10.1±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>11.3±1.3</td>
<td>12.2±0.9</td>
<td>11.5±1.2</td>
<td>T vs. B, F vs. T</td>
</tr>
<tr>
<td>Base excess [mmol/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>3.5±0.9</td>
<td>2.2±1.6</td>
<td>2.1±1.8</td>
<td>F vs. B</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>3.6±1.3</td>
<td>1.4±2.1</td>
<td>3.4±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>3.5±1.0</td>
<td>0.3±1.0</td>
<td>1.1±3.5</td>
<td>F vs. T</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>4.7±2.3</td>
<td>1.0±1.4</td>
<td>3.0±4.7</td>
<td>F, T vs. B</td>
</tr>
<tr>
<td>P-Lactate [mmol/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>2.9±0.9</td>
<td>3.6±1.2</td>
<td>3.6±1.4</td>
<td>NS</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>2.5±0.3</td>
<td>3.7±0.9</td>
<td>1.9±0.4</td>
<td>T vs. B, T vs. F</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>3.0±0.8</td>
<td>5.7±1.0</td>
<td>7.9±2.0</td>
<td>F, T vs. B, F vs. T</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>2.6±0.4</td>
<td>4.8±0.5</td>
<td>4.4±2.4</td>
<td>T vs. B</td>
</tr>
<tr>
<td>P-AST [µkat/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>1.2±0.1</td>
<td>1.8±0.3</td>
<td>2.4±0.5</td>
<td>F, T vs. B, F vs. T</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>1.6±0.2</td>
<td>1.8±0.3</td>
<td>2.4±0.8</td>
<td>F vs. B, F vs. T</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>1.4±0.2</td>
<td>2.6±0.9</td>
<td>4.1±1.5</td>
<td>F vs. B, F vs. T</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>1.6±0.3</td>
<td>2.6±1.6</td>
<td>5.4±3.8</td>
<td>F vs. B</td>
</tr>
<tr>
<td>P-Urea [mmol/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>8.2±0.9</td>
<td>10.1±2.3</td>
<td>10.5±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>7.6±1.0</td>
<td>9.5±2.6</td>
<td>10.1±2.1</td>
<td>F vs. B, T</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>7.7±0.8</td>
<td>12.7±1.9</td>
<td>19.8±2.6</td>
<td>F vs. B, F vs. T</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>8.2±1.0</td>
<td>11.7±2.3</td>
<td>16.9±3.2</td>
<td>F, T vs. B, F vs. T</td>
</tr>
<tr>
<td>P-Creatinine [µmol/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Saline</td>
<td>24±5.7</td>
<td>28±8.5</td>
<td>35±7.7</td>
<td>F vs. B</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>17±1.5</td>
<td>25±4.7</td>
<td>26±6.3</td>
<td>F, T vs. B</td>
</tr>
<tr>
<td>3: LPSIL + Saline</td>
<td>22±4.0</td>
<td>39±13</td>
<td>69±24</td>
<td>F, T vs. B, F vs. T</td>
</tr>
<tr>
<td>4: LPSIL + GE</td>
<td>20±4.2</td>
<td>35±7.5</td>
<td>54±21</td>
<td>F, T vs. B, F vs. T</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±standard deviation for $n=7$ rats in each group. C, saline-challenged controls; LPSIL, lipopolysaccharide/IL-2-challenged rats; saline, saline-infused rats; GE, gentamicin-infused rats. a) Significant within-group differences ($p<0.05$), NS, nonsignificant. b) Significant differences ($p<0.05$) between groups are indicated by arabic numerals.
GE. Another study provided contradictory evidence of a 30% lower $CL_{cr}$ in non-septic rats after a single-dose administration of 4 mg GE i.v. Notably, the authors have measured urinary excretion of creatinine over an interval of 0–24 h and serum creatinine at 24 h after administration of GE. In this way, a time-averaged value of $CL_{cr}$ was calculated over a time interval much longer than that required for a 97% decrease in the GE serum concentration (3–5 h).

The value of the total plasma $CL$ of GE obtained in the present study for nonseptic rats agrees well with previous reports. Despite the fact that both $CL_{cr}$ and renal $CL$ of GE were markedly reduced in LPSIL-2 rats, the value of total plasma $CL$ during the treatment period was only marginally less due to a dominant contribution of nonrenal mechanisms in this group. Thus, the general view that the rate of GE elimination is determined mainly by GFR is challenged in this rat model of early sepsis. Bergeron et al. have studied the pharmacokinetics of GE as well as several parameters of renal function and histology after a single bolus i.v. dose of 10 mg/kg GE administered to rats treated with a low-dose LPS (E. coli O127:B8, a 15-min i.v. infusion of 0.25 mg/kg). Unlike in the present study, however, GE-free controls and endotoxemic rats were not included. Consequently, the effect of GE could not be separated from that of LPS. In agreement with our findings, endotoxemic animals showed a similar drop in GFR (inulin clearance) and renal $CL$ of the drug, while the total plasma $CL$ remained unchanged at 3 h following LPS.

Table 2. Characteristics of Renal Function

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: C+Saline</td>
<td>20.7±6.5</td>
<td>20.3±10.9</td>
</tr>
<tr>
<td>2: C+GE</td>
<td>23.6±11.5</td>
<td>14.0±3.3</td>
</tr>
<tr>
<td>3: LPSIL+Saline</td>
<td>18.9±6.8</td>
<td>15.3±6.4</td>
</tr>
<tr>
<td>4: LPSIL+GE</td>
<td>26.7±12.4</td>
<td>16.0±13.2</td>
</tr>
</tbody>
</table>

Table 3. Pharmacokinetics of Gentamicin

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 2: C+GE</th>
<th>Group 4: LPSIL+GE</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>After i.v. bolus $C_{max}$ [mg/L]</td>
<td>10.7±2.2</td>
<td>9.0±2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>$V_c$ [L/kg]</td>
<td>0.22±0.025</td>
<td>0.34±0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>During and after i.v. infusion $C_{max}$ [mg/L]</td>
<td>7.5±2.7</td>
<td>9.6±2.1</td>
<td>0.1</td>
</tr>
<tr>
<td>$CL$ [mL/min·kg$^{-1}$]</td>
<td>6.7±1.1</td>
<td>5.9±1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>$CL_{u}$ [mL/min·kg$^{-1}$]</td>
<td>3.8±0.53</td>
<td>2.2±0.59</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$CL_{u}/CL$</td>
<td>0.60±0.13</td>
<td>0.51±0.35</td>
<td>0.5</td>
</tr>
<tr>
<td>$t_{1/2}$ [min]</td>
<td>23.8±5.8</td>
<td>42.3±20.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>180 min after the end of i.v. infusion $C_{\text{influx}}$ [mg/L]</td>
<td>1.0±0.39</td>
<td>2.5±2.0</td>
<td>0.09</td>
</tr>
<tr>
<td>GE in the kidney tissue homogenate [mg/L]</td>
<td>1.6±0.68</td>
<td>3.5±2.2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±standard deviation for $n=7$ rats in each group. C, saline-injected controls; GFSIL, lipopolysaccharide+IL-2-infused rats; saline, saline-infused rats; GE, gentamicin-infused rats. a) Paired t-test of within-group differences. b) Significant differences ($p<0.05$) between groups are indicated by arabic numerals.
and animal studies have repo
rat rates 5 to even 10 times
duce systemic hypotension and a drop in GFR, whereas lower
(LPS, live attenuated bacteria, cecal ligation and puncture),
homogenate from LPSIL-2-challenged rats, which agrees with
supportive therapy and the timing of measurements. Most
affected as an i.v. bolus\textsuperscript{22}) or 150 µg/kg per hour LPS given in a
continuous i.v. infusion.\textsuperscript{23} In contrast to a marked and sus-
tained $CL_{cr}$ decrease in the groups injected with LPSIL-2, no
between-group differences were observed in the rate of urine
flow. Other researchers have observed only a transient drop in
the flow of urine collected from Wistar rats after a bolus dose
of 1 mg/kg LPS, with a return to a baseline value by 150 min
having also been observed.\textsuperscript{22} The rate of urine flow was un-
affected by a GE infusion rate of 0.05 mg/kg per min. Similar
findings were made in Sprague Dawley rats after GE infusions
at rates 5 to even 10 times higher.\textsuperscript{29}

Changes in renal function parameters observed in the early
period following LPSIL-2 administration were comparable to
those described by others after 1 mg/kg of LPS adminis-
tered as an i.v. bolus\textsuperscript{22} or 150 µg/kg per hour LPS given in a
continuous i.v. infusion.\textsuperscript{23} In contrast to a marked and sus-
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at rates 5 to even 10 times higher.\textsuperscript{29}

Previous studies, which focused on the time-dependent
changes of urinary markers from the onset of sepsis, provided
heterogeneous results due to the influence of several factors,
such as the animal model used, the mechanism of injury
(LPS, live attenuated bacteria, cecal ligation and puncture),
supportive therapy and the timing of measurements. Most
researchers have used the fractional excretion of sodium as a
marker of tubular function. Experience with $FE_{UN}$ is, however,
relatively limited.\textsuperscript{8,24,25} Both indices increase in acute renal
failure caused by tubular necrosis. Both renal hypoperfusion
as well as septic hyperdynamic kidney injury cause a reduc-
tion in filtration pressure and GFR (in the latter case mainly
due to effenter arteriolar vasodilatation). Consequently, the
renin-angiotensin-alderosterone system (RAAS) and other ho-
meostatic mechanisms are activated. The increased reabsorp-
tion of sodium, water and urea results in decreased values of
$FE_{Na}$ and $FE_{UN}$.\textsuperscript{16,24,25} The gradual decrease in GFR ob-
served in rats injected with LPSIL-2 during the treatment and
follow-up periods was followed by a drop in $FE_{UN}$ during the
latter period, which is a sign of a preserved tubular compensa-
tory mechanism. In line with this, the renal histopathology did
not reveal tubular necrosis or other significant sepsis-induced
damage. There was no sign of renal microvascular leak-
age. We observed neither extravascular fluid expansion nor
albumin-bound indocyanine green in the interstitium. Accord-
ing to a recent systematic review, a limited number of human
and animal studies have reported histopathological findings of
septic acute kidney injury. Among these studies, only about
20% have found acute sepsis-induced tubular necrosis.\textsuperscript{24}

Low-dose LPS was selected in order to mimic early sepsis
without severe hemodynamic compromise. Higher doses in-
duce systemic hypotension and a drop in GFR, whereas lower
doses decrease GFR without major changes in blood pressure
and associated multi-organ injury.\textsuperscript{22,23} Low-dose IL-2 has
been considered a mediator and enhancer of capillary leakage
in early sepsis without immediate worsening of cardiovascular
functions.\textsuperscript{26} This results from a cascade of events leading
to induction of NO synthesis and release which, directly or
indirectly, injures capillaries and causes leakage.\textsuperscript{11,12} Endotox-
emia resulted in moderate increases in respiratory and heart
rates. Changes in acid-base parameters corresponded to the
development of metabolic acidosis compensated by hyperven-
tilation leading to hypocapnia. Results of clinical chemistry
investigations confirmed the changes typical for endotoxemia
in rodents and large animals as well as in septic patients, i.e.,
elevated concentrations of plasma lactate, AST, creatinine and
urea. In our model of early sepsis, the lung and spleen were
the organs affected, while the morphology of other organs
(kidney, liver, heart, brain, intestine and inner ear) was nor-
mal. All 25 rats investigated in the preliminary experiment
survived the dose of 1 mg LPS combined with 15 µg per kg
of IL-2. In contrast, an intra-peritoneal injection of E. coli
055:B5 LPS at a dose of 10 mg/kg resulted in mortality rates of
60% and 75% in young and old Wistar rats, respectively,
and the mortality remained as high as 50% in both age cate-
gories after a dose of 5 mg/kg.\textsuperscript{29} Unlike in the present study,
the total plasma CL of GE (3 mg/kg, i.v.) dropped by 59% and
87% in young adult (2–3 months) and old (22–24 months) rats,
respectively, at 24 h following the injection of 5 mg/kg of LPS.

The model presented here has its limitations and extrapo-
lation to clinical conditions should be done with caution. It is
worth noting that most innovative therapeutic interventions
that improved morbidity and mortality in animal models of
sepsis failed in clinical trials.\textsuperscript{27} Human sepsis is a complex
disease characterized by the elevation of circulating inflam-
matory cytokines (systemic inflammatory response syndrome),
endothelial activation, hemodynamic changes (warm shock
followed by cold shock), dysregulation of coagulation and,
subsequent multi-organ failure. It occurs more frequently in
elderly and immunocompromised patients. Injection of LPS
to rodents reproduces only some consequences of sepsis in
humans. Large animal or primate models using cecal ligation
and puncture or bacteria injection are more informative in this
regard.\textsuperscript{21} Septic patients develop diffuse microvascular leak
and tissue edema. Injection of LPS+IL-2 to rats also resulted in
capillary leakage as documented by laboratory tests and
pharmacokinetic results. These features of sepsis have not
been thoroughly investigated in animal models and the present
model offers such possibility. Other findings which resemble
early sepsis in patients were tachycardia, tachypnea, a de-
creased GFR, the absence of significant kidney hypoperfusion
and tubular dysfunction, lactate acidemia, elevations of urea,
creatinine and AST.

CONCLUSION

The major characteristics of the early sepsis model induced
in Wistar rats by a low-dose LPS and IL-2 are as follows:
 systemic microvascular leakage, a drop in GFR with only
partially preserved tubular compensatory mechanisms,
unchanged renal morphology, and histologically proven pulmo-
nary injuries and splenic alterations-signs of activation. In the
range of therapeutic steady-state concentrations maintained
over 3h, GE does not reduce $CL_{cr}$ (the marker of GFR) either in
nonseptic or LPSIL-2 rats. This is in contrast to its marked
decrease due to the effect of LPSIL-2 alone. An enlarged $V_C$ and $V_D$ along with a drop in renal CL of GE proportional to that of GFR are the remarkable changes of GE disposition in this rat model of early sepsis which correspond to those occurring in septic patients. The model may thus enable to study the effects of interventions aimed to modify pathophysiological processes that significantly influence pharmacokinetics and dynamics of aminoglycosides and possibly of other antibiotics in sepsis. Nonrenal elimination routes which work to compensate the reduced renal CL of GE in septic rats deserve further study.

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